

THE GENETIC STRUCTURE OF *Heliconius erato* POPULATIONS (LEPIDOPTERA; NYMPHALIDAE)

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ABSTRACT

Samples of butterflies (*Heliconius erato*, Nymphalidae) were drawn from five different localities in Rio Grande do Sul (Brazil) to study allozyme polymorphisms and genetic structure. Four loci were investigated, Pgm, Lap, Mdh1 and Mdh2 (the latter being monomorphic). The five localities, which were less than 30 km apart, showed a high genotypic similarity, ranging from 0.86 to 0.95. However, they also showed a significant isolation component when considered as part of a larger population ($F_{ST} = 0.0247$; $P < 0.001$). When they were taken as forming only two subdivisions of a larger population, F_{ST} was even greater, 0.0798 ($P < 0.001$). The component due to inbreeding (F_{IS}) was also significant in both cases, being respectively 0.1859 ($P < 0.05$) and 0.2153 ($P < 0.001$). These results confirm the island nature of *H. erato* populations previously suggested on the basis of ecological information. The high levels of inbreeding could be associated with certain behavioural traits shown by these insects.

INTRODUCTION

Although the butterflies of the genus *Heliconius* have been examined with a variety of approaches (revisions in Brown Jr., 1981; Turner, 1981), few attempts have been made to study the genetic variability of natural populations as well as the way such variability is organized (Turner *et al.*, 1979; Lima and Araújo, 1982; Mallet, 1984). Our investigation was designed to assess the contribution of the spatial pattern of natural populations of *Heliconius erato phyllis* in the organization of the variability due to structural genes.

Traditionally two models of population structure are considered, both due to Sewall Wright: the island model and isolation by distance. The first assumes that "the population is divided into groups that are panmictic within themselves, except for the reception of small proportions of immigrants, representative of the population as a whole" (Wright, 1969). In the isolation by distance model,

immigrants come from neighboring populations: the closer ones exchanging more individuals, and so on. A general treatment of the island model leads to a consideration of at least a three-level hierarchy of entities: total population, demes, and individuals within demes. The analysis of such a structure is commonly made by the F-statistic, in which the homozygosity of an individual in the total population can be partitioned into components within demes, F_{IS} , and among demes F_{ST} . This is the approach used in the present paper.

MATERIAL AND METHODS

Samples of butterflies were gathered from five localities around Porto Alegre (30°04'S; 51°11'W), the capital of the state of Rio Grande do Sul (Brazil): Morro Sapucaia (MSP), Sapucaia (SAP) and Águas Belas (ABE), each about 30 km from Porto Alegre; Instituto de Pesquisas Hidráulicas (IPH) and Morro Santana (MSA), both within the Porto Alegre limits (Figure 1). The butterflies were captured in the Spring and early Summer of 1986, placed in polystyrene boxes to keep them alive and then brought to the laboratory. There they were frozen at -10°C until preparation for electrophoresis. The systems studied were phosphoglucosmutase (PGM EC 2.7.5.1), NAD-dependent

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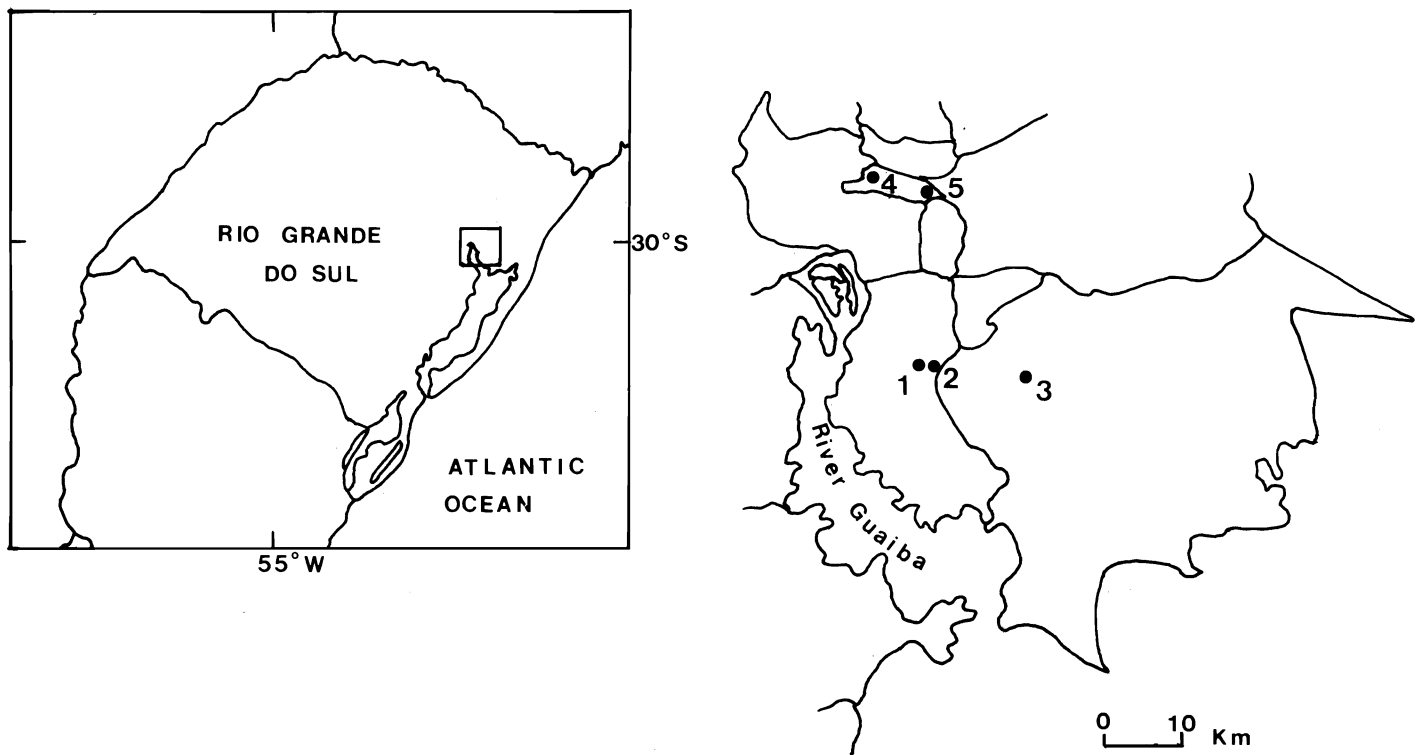


Figure 1 - Localities where the samples were made. 1 = IPH; 2 = MSA, both within the Porto Alegre district; 3 = ABE, Viamão district; 4 = SAP; 5 = MSP, both within Sapucaia do Sul district. See Material and Methods for abbreviations.

malate dehydrogenase (MDH EC 1.1.1.37) which were prepared after Harris and Hopkinson's (1976) method and leucine aminopeptidase (LAP EC 3.4.11.1), prepared following Brewer and Sing's (1970) method. We first tested the techniques using head, thorax and abdomen of the insects, one at a time. Although all three segments showed good and similar zymograms we decided to use only the abdomen as it was easiest to work with. MDH and LAP were studied on horizontal 11% starch gel, and PGM on a 5% acrilamide gel. The abdomens were homogenized individually in test tubes surrounded by ice and centrifuged at 3,000 rpm for 15 minutes; the supernatants were applied to the gel with a Whatmann no. 3 filter paper. Electrophoresis was run for 18 hours at 4°C.

Tests of Hardy-Weinberg equilibrium for each sample were made by comparing observed and expected heterozygosities to obtain the "d" quotient of Bailey (1974). Testing individual genotype frequencies would lead in many cases to an excess of null frequencies and/or small expectations. To compare the allele frequency distributions among the samples, the "lambda" statistics of Shields and Heeler (1979) was applied, with a computer program developed by one of us (L.M.S.). The components of the F-statistics were estimated according to Weir and Cockerham (1984) and their significance tested with chi-square following Li and Horvitz (1953) and Workman

and Niswander (1970). Estimates of genotypic identity as well as of the probability of unique genotype were made as suggested by Hedrick (1971).

RESULTS

Sample size and enzyme pattern

The number of butterflies sampled for each system is indicated in Table I. Sampling from Morro Santana was regrettably poor, while the low number for LAP from Águas Belas was due to an accidental loss. For the remaining systems and places, sample sizes were satisfactory,

Table I - Number of individuals sampled for each enzyme system and population.

Enzyme	ABE	IPH	MSP	SAP	MSA
PGM	46	70	29	47	9
MDH1	38	81	34	34	9
MDH2	36	81	34	48	9
LAP	4	26	21	34	8

ABE = Águas Belas; IPH = Instituto de Pesquisas Hidráulicas; MSP = Morro Sapucaia; SAP = Sapucaia; MSA = Morro Santana.

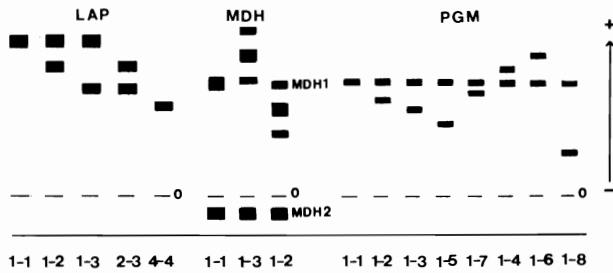


Figure 2 - Schematic representation of the electrophoretic pattern showed by the four enzymatic loci used in the present study. Further explanations in the text.

corresponding to at least 50% of the effective size of *H. erato* populations (Oliveira and Araújo, 1992).

Figure 2 shows the general zymogram pattern for the three systems studied. LAP and PGM are monomeric enzymes with four and eight alleles respectively; MDH is a dimeric one. This latter system showed an anodic component (MDH1, three alleles) and a cathodic one (MDH2, monomorphic).

Gene and genotype frequencies

The number of detected alleles differed in the populations; for instance, PGM had five alleles detected at Águas Belas, seven at Instituto de Pesquisas Hidráulicas and Sapucaia, six at Morro Sapucaia and only four at Morro Santana (Table II). The distributions of allelic frequencies were not significantly different according to the "lambda" statistics, although the differences observed for LAP are on the borderline. For each system the values obtained were: PGM, "lambda" = 17.53; 17 d.f.; $0.3 < P < 0.5$; MDH1, "lambda" = 4.03; 4 d.f.; $0.2 < P < 0.3$; LAP, "lambda" = 14.09; 8 d.f.; $0.05 < P < 0.10$. Due to the different number of alleles at each location, for the same system, values of H(m), maximum heterozygosity in equilibrium, are not the same.

Tests for Hardy-Weinberg equilibrium (excluding Morro Santana, due to the small sample size) demonstrated significant differences for LAP from the Instituto de Pesquisas Hidráulicas and for Morro Sapucaia (Table II). According to Weir (1990) it is possible to determine which of the heterozygote genotypes do not follow the expectations by using a chi-square with one degree of freedom. For Instituto de Pesquisas Hidráulicas, the frequencies of the observed heterozygotes were: 1-2 = 4%; 1-3 = 15%; 2-3 = 8%. Only 1-2 showed a significant difference (observed less than expected; $\chi^2 = 6.63$, $P < 0.01$). In Morro Sapucaia, only the heterozygotes 1-2 (10%) and 1-3 (5%) were detected; again, genotype 1-2

Table II - Allele frequencies of the three enzyme systems in the populations sampled. H(o), H(e), H(m) observed, expected and maximum heterozygosity in equilibrium, respectively.

Enzyme	Alleles	ABE	IPH	MSP	SAP	MSA
PGM	1	0.44	0.54	0.62	0.62	0.50
	2	0.32	0.21	0.03	0.10	0.22
	3	0.06	0.05	0.02	0.04	0.06
	4	0.17	0.16	0.28	0.20	0.22
	5	0.01	-	0.02	-	-
	6	-	0.02	0.03	0.03	-
	7	-	0.01	-	0.01	-
	8	-	0.01	-	-	-
	H(o)	0.54	0.63	0.58	0.55	0.89
	H(e)	0.66	0.62	0.50	0.56	0.65
H(m)	0.80	0.88	0.83	0.83	0.75	
MDH1	1	0.92	0.97	0.96	0.97	1.00
	2	0.03	0.01	-	-	-
	3	0.05	0.02	0.04	0.03	-
	H(o)	0.10	0.05	0.09	0.06	-
	H(e)	0.15	0.06	0.09	0.06	-
	H(m)	0.67	0.67	0.50	0.50	-
LAP	1	0.88	0.36	0.74	0.59	0.67
	2	0.12	0.33	0.14	0.31	0.33
	3	-	0.27	0.02	0.10	-
	4	-	0.04	0.10	-	-
	H(o)	0.25	0.27	0.14	0.47	0.26
	H(e)	0.21	0.69	0.42	0.55	0.44
	H(m)	0.50	0.75	0.75	0.67	0.50

* Significant at the 5% level.

**Significant at the 1% level.

was at a low frequency compared to that expected, the difference being nearly significant ($\chi^2 = 3.61$, $0.05 < P < 0.10$).

Genetic similarity

The genetic similarity between the populations was assessed by the genotypic identity and the probability of a unique genotype. There was a great similarity among the populations (Table III). As for unique genotypes, greater values were found for the Instituto de Pesquisas Hidráulicas population (Table III).

Hierarchical structure of the populations

With the three loci taken together the effect of subdivision on the genotypic frequencies were highly

Table III - Genotypic identity (upper triangle) and probability of a unique genotype (lower triangle) for the populations sampled, averaged over all loci.

	ABE	IPH	MSP	SAP	MSA
ABE	-	0.95	0.92	0.95	0.89
IPH	0.04 0.21	-	0.86	0.90	0.87
MSP	0.15 0.09	0.13 0.01	-	0.94	0.91
SAP	0.05 0.12	0.03 0.01	0.04 0.06	-	0.90
MSA	0.14 0.04	0.20 0.00	0.14 0.04	0.12 0.00	-

For the lower triangle, the upper number refers to the pair member at the top of the matrix and the lower number to the pair member at the side.

significant and the component due to inbreeding (Table IV). This latter value represents more than 90% of the contribution to F_{IT} ; homozygosity seems to be mainly due to inbreeding.

Although the populations are geographically close, they can be analysed as if they were subdivided into two groups: one comprising Morro Sapucaia and Sapucaia with a distance between them of about 10 km, now treated as a single deme (points 4 and 5 in Figure 1), and the other Águas Belas, Instituto de Pesquisas Hidráulicas and Morro Santana with an average distance of 15 km, being the other deme (points 1, 2, 3, Figure 1). The results of this new hierarchy are shown in Table V. Again, F_{IS} and F_{ST} are statistically significant for the total number of loci. Moreover, the value ascribed to inbreeding is very similar to the one shown in Table IV, its contribution to F_{IT} being 77%.

DISCUSSION

The five populations showed a high degree of similarity, as expressed by their allele frequencies and by the genotypic identity and probability of unique genotypes

Table IV - Components of the F-statistics for the five populations sampled considered as part of a larger population.

	F_{IS}	χ^2	F_{ST}	χ^2	d.f.	F_{IT}
LAP	0.2753	7.0584	0.0173	9.6534	12	0.2878
MDH1	0.5864	67.3975***	0.0994	77.9296***	8	0.6275
PGM	0.0630	0.7978	0.0149	41.9286*	28	0.0769
Total	0.1859	75.2438*	0.0247	129.5116***	48	0.2061

*Significant at the 5% level.

***Significant at the 0.1% level.

d.f. - Degrees of freedom.

Table V - Components of the F-statistics considering ABE - IPH - MSA as one population and MSP - SAP as another.

	F_{IS}	χ^2	F_{ST}	χ^2	d.f.	F_{IT}
LAP	0.3040	8.5947*	0.1582	88.2756***	3	0.4141
MDH1	0.6693	87.8006***	0.0904	70.8736***	2	0.6992
PGM	0.0610	0.7479	0.0207	58.2498***	7	0.0805
Total	0.2153	97.1432***	0.0798	217.3990***	12	0.2779

*, ***, d.f. - Same as in Table IV.

(Tables II and III). The populations from IPH and MSP showed significant deviations from the expected proportions for the 1-2 heterozygote of leucine-amino-peptidase. It is difficult to interpret this finding in the absence of more information on other variables, especially since the sample sizes for that particular system were small (Table I).

Though this survey was not designed to study the amount of polymorphism (P) detected in populations, or the average heterozygosity per individual (H), the data are similar to previous reports. Turner *et al.* (1979) found 47-76% for P (three samples; 17 allozyme loci) and 24% for H in *H. erato*; the corresponding values in our study were 75% and 22-29% respectively. They estimated Nei's genetic identity in the range 0.93-0.99; our values of Hedrick's genotype identity were 0.86-0.95, which, translated to Nei's statistics gives 0.93-0.99.

The *H. erato* populations showed a moderately high degree of isolation whatever the type of hierarchy considered: if the five samples are taken as part of a large population, then $F_{ST} = 0.0247$ (Table IV); if they are grouped in two units, according to geographic proximity, then F_{ST} between the two units equals 0.0798 (Table V). This emphasizes the island nature of the populations; the closer they are to each other, more homogeneous they appear. The use of protein polymorphism to investigate the genetic structure of natural populations of *H. erato* confirmed previous studies that used capture-recapture for an analysis of dispersal (Saalfeld and Araújo, 1981; Pansera and Araújo, 1983; Di Mare and Araújo, unpublished results): this butterfly has a remarkable phylopatry (although Mallet, 1984, argues the contrary).

The contribution of inbreeding to total homozygosity was very high (Tables IV and V). Similar values have been obtained using different approaches (Di Mare and Araújo, 1986; Oliveira and Araújo, 1992). This high average inbreeding coefficient could facilitate the evolution of some behavioural adaptations found in *H. erato*. They roost gregariously, using the same place for months if undisturbed (Gilbert, 1975). Santos (unpublished results) studied the behaviour of *H. erato* in captivity and was able to show that by dividing the life span of the butterflies into two halves, they are significantly more faithful to the roosting site in the second half. The same author investigated the cannibalism towards eggs and noted that there was a significant decrease when the egg was of a relative (sibling) of the larva. The possibility of kin selection, long ago stressed by Benson (1971) to explain the evolution of unpalatability in these butterflies cannot then be ruled out. Both roosting and selective cannibalism could evolve more rapidly if the members of a group were relatives. On the other hand, from a populational view, high inbreeding could facilitate the removal of deleterious mutations.

ACKNOWLEDGMENTS

Thanks are due to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) for the financial support given to our research.

RESUMO

Foram feitas amostras de borboletas (*Heliconius erato*, Nymphalidae) de cinco diferentes locais do Rio Grande do Sul (Brasil) para verificação de polimorfismos enzimáticos e da estrutura genética. Quatro locos foram investigados, Pgm, Lap, Mdh1 e Mdh2 (este último, monomórfico). As cinco localidades, cuja distância entre si não ultrapassa os 30 Km, mostraram uma alta similaridade genotípica, variando de 0.86 a 0.95; entretanto elas apresentaram um significativo componente devido ao isolamento, quando consideradas como fazendo parte de uma grande população ($F_{ST} = 0.0247$; $P < 0.001$). Quando elas foram tomadas como formando apenas duas subdivisões de uma população maior, F_{ST} foi ainda maior, 0.0798 ($P < 0.001$). O componente devido ao endocruzamento (F_{IS}) também foi significativo em ambos os casos sendo, respectivamente, 0.1859 ($P < 0.05$) e 0.2153 ($P < 0.001$). Estes resultados confirmam a natureza do tipo ilha das populações de *H. erato*, previamente sugerida com base em informações ecológicas; mais ainda os seus altos níveis de endocruzamento são explicados como uma possível adaptação para a evolução de certas características comportamentais.

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(Received March 29, 1993)